INCIDENCE OF SHIKATA CELL (HBsAg) IN CIRRHOSIS LIVER CASES OF BUNDELKHAND

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DOCTOR OF MEDICINE
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C E R T I F I C A T E

This is to certify that the research work entitled "INCIDENCE OF SHIKATA CELL (HBsAg) IN CIRRHOSIS LIVER CASES OF BUNDELKHAND" which is being submitted as THESIS for M.D.(Medicine) examination of Bundelkhand University, 1984, by Dr. Sudhir Mehrotra has been carried out in the Department of Medicine. He has put in the necessary stay in the department as per University regulation.

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CERTIFICATE

Certified that the present research work entitled "INCIDENCE OF SHIKATA CELL (HBsAg) IN CIRRHOSIS LIVER CASES OF BUNDELKHAND" was conducted by Dr. Sudhir Mehrotra under our guidance and supervision. The techniques and statistics mentioned in the thesis were actually undertaken by the candidate himself.

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C O N T E N T

INTRODUCTION

INTRODUCTION

Cirrhosis of the liver is a known clinical entity since ancient times. It was Laennaec who coined the term cirrhosis and described it in detail in 19th century. A number of factors have been highlighted as the cause of cirrhosis from time to time. Sherlock and others recognised the role of infective hepatitis in genesis of post-necrotic cirrhosis.

The variation in pattern and aetiology of cirrhosis liver in different geographical regions have been well documented by a number of studies throughout the world. Basu (1967) reported incidence of 55% of post-necrotic cirrhosis in India, which is quite high as compared to Western countries. On the contrary, alcoholic cirrhosis forms the major bulk in Western countries while it is rare in our country.

The major breakthrough in hepatitis research came after a stalemate of many years through the discovery of Australia antigen by Blumberg et al in 1965. Now there is vast literature on the relationship of Australia antigen (HBsAg) and

chronic hepatic diseases (Fox et al., 1969; Faber et al., 1970; Dudley et al., 1972 and Sherlock et al., 1975). The progression from acute hepatitis through chronic hepatitis to cirrhosis is well documented in literature (Shaefor et al., 1967) and later on association of Hepatitis B surface antigen (HBsAg) with cirrhosis liver (post necrotic, mixed and cryptogenic) was described by Sherlock et al (1970).

The incidence of HBsAg with cirrhosis liver is also variable in different geographical regions. The positivity is 26.6% in U.S.A. (Wright et al., 1969); 18% for Denmark (Faber et al., 1970) and 59.2% (Sama et al., 1974) for India.

Hitherto the localization of HBsAg, has remained a complicated procedure requiring Immunofluorescence, Immunoperoxidase precipitation, Agar gel diffusion, Counter-electro phoresis, Electronmicroscopy or Radioimmunoassay, which were expensive, time consuming and remained a rider regarding frequent studies from different areas. It was in 1974 when Shikata et al first

observed accidently staining of hepatocytes by Von Geison's stain for elastic tissue.

This later on paved the way for the development of simple Orcein and other stains for demonstration of HBsAg in biopsies of chronic liver diseases. The technique is simple, sensitive, reproducible and comparable to any other specialized standard technique. It is not only effective in fresh liver biopsy but also in stored liver biopsies and so a prospective and retrospective study can be carried out at any centre.

Although the incidence of post necrotic cirrhosis is high in India as reported by various workers, but there is paucity of literature from Indian scene regarding the incidence of Shikata cell (HBsAg) in cirrhosis. The study was aimed to work out the incidence of Shikata cell (HBsAg) using modified orcein technique in cirrhosis liver cases in Bundelkhand region, where the incidence of cirrhosis seems to be much higher than other regions.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

The Cirrhosis of liver is a well known clinical entity since ancient times. Eristitratus of Alexandaria was able to recognise it when stony, hard, hobnailed liver was accompanied with dropsy. The term cirrhosis was coined by Laennac in 19th century. The word cirrhosis is derived from Greek 'Kirros', meaning tawny because the "projecting nodules were of fawn or yellowish russel bordering on the greenish." The cannotation of the word has now completely changed. The essence of cirrhosis is a disturbance of lobular archietecture with the formation of regenerative nodules and connective tissue septa.

The fifth Pan American Congress of Gastroentrology, Havana (1956) arrived at anatomical defination of cirrhosis and consensus on the clinical concepts relating to the disease.

Anatomical defination:-

(a) Involvement of all parts of the liver without necessarily affecting each lobule.
(b) Presence of cellular necrosis at some stage of the disease.
(c) Nodular parenchymal regeration.
(d) Diffuse fibrosis and
(e) Disorganisation of the lobular archietecture with connective tissue bands uniting centrilobular zones with the

portal tracts.

Clinical aspects:-

(a) Chronic disease

(b) Liver cell failure and

(c) Portal hypertension of variable severity.

The cirrhosis is classified morphologically into three anatomical types micronodular (regular monolobular), macronodular (irregular multi-lobular) and mixed.

Pathologist classified cirrhosis into portal cirrhosis, post necrotic cirrhosis, biliary cirrhosis, cardiac cirrhosis and alcoholic cirrhosis, so as to provide an aetiological significance to each type. As the pathologic pattern may represent nonspecific hepatic response to many different forms of liver cell injury, while a variety of morphological pattern may be produced by a specific aetiology in a single patient, so this classification do not hold good.

While adopting a clinical classification of cirrhosis, the clinical signs and symptoms may not reflect accurately the extent and precise

nature of the cirrhotic process or its stage of activity. Although till date no satisfactory clinical, aetiologic or morphologic classification of cirrhosis is available, but it is possible to categorize most case of cirrhosis clinically adding qualifying morphologic or aetiologic term when possible.

The factors leading to cirrhosis comprise a long and exhaustive lists which include :

a) Virus hepatitis - Infectious hepatitis
- Serum hepatitis

b) Alcholism

c) Prolonged cholestasis

d) Cardiac failure

e) Hepatic venous obstruction

f) Haemachromatosis

g) Hepatolenticular degeneration

h) Fibrocystic disease

i) Galactosaemia

j) Congenital syphilis

k) Drugs like - methyldopa, methotrexata

l) Chemical poisons - Arsenic, Phosphorus

m) Plant poisons - Aflatoxins
- Crotolaria
- Senecia and Heliotropism.

n) Worm infestation - clonorchiasis

o) Disturbed immunity.

The first reference to epidemic jaundice has been ascribed to Hippocrates. The earliest record in Western Europe is in a letter written in A.D.751 by Pope Zacharis to St. Boniface Archibishop of Mainz. Epidemic jaundice had been a problem in Franco Prussian Ward. The American Civil War, World Wars I and II and lastly in 1955 in Delhi. In this a remarkable epidemic followed, flood and diversion of a river which heavily contaminated the city water with the causative, an infectious agent leading to acute inflamation of the liver.

Although it has been known for a century that hepatitis was often associated with faecally contaminated material and that percutaneous infection could result in another form of hepatitis. It was not until 1951, when Mac Callum showed by means of series of feeding and innoculation experiments that there were at least two viral agents producing similar diseases but there was no cross immunity between the two. The two agents

produced their disease after different incubation periods. The one had maximum infectivity by mouth and the other by percutaneous injection. The author suggested that the orally transmitted shorter incubation agent be called hepatitis A Virus(HAV) and the parentally transmitted longer incubation one hepatitis B (HBV).

The major break through in hepatitis research came after a stalemate of many years by the discovery of Australia antigen by Blumberg et al in 1965. It is now a general agreement that Australia antigen is associated only with large incubation serum hepatitis or virus B hepatitis and not with infectitous hepatitis or virus A hepatitis (WHO 1973). However, in later years the other modes of transmission have also been implicated. Some of them are: Sexual (Hersh et al 1971, Henigist et al 1973). Kissing (Villarejos et al 1974), Human biting (Mac Quarrie et al 1974) and transplacental (Stevens et al 1975). In the subsequent years, the structure of virus A was studied and defined. The patient who suffered from a yet another virus, different from A and B type, was classified as Non A, Non B, type virus.

The hepatitis B antigen is an immunological marker of hepatitis B virus (HBV). The antigen is a macromolecule and has predominantly protienous material with varying amount of lipids. The antigen is stable morphologically and immunologically and is not affected by repeated freezing and thawing or heating at 56° overnight or at 60°c for 1 hour; but is destroyed by heating at 65-100°c (Gerin et al 1969). Electromicroscopy studies of serum containing this antigen revealed three different particles. The most numerous are the 22 nm particles, which appear as spherical or long filamentous form varying in length up to 300 nm, these are antigenically identical with the outer surface or coat of the Hepatitis B virus and are thought to represent the excess viral coat protein. Less frequently seen in Serum are large 42 nm spherical Dane particles which are believed to represent intact hepatitis B virus (HBV). These large particles have characteristically an outer coat and inner icosahedral core measuring 27 nm in diameter. (Bayer, Blumberg and Werner 1968, Almedia et al 1969; Dane, Cameron and Briggs, 1970). Almedia, Rubanstein and Stott (1971) showed that this central core was immunologically distinct

from its coat and the small spheres and tubules and the antigen is referred to as the hepatitis & core antigen (HBcAg) and the corresponding antibody is anti HBC. Within the core particle is a predominantly double stranded DNA which is associated with a DNA polymerase.

The long tubuler and small spherical particles 22 nm. Originally called as Australia antigen or hepatitis associated antigen was subsequently referred as the hepatitis B surface antigen (HBsAg). This HBsAg is also present on the surface of the 42 nm Dane particles. There is a common group reactive antigen, shared by all HBsAg positive serums. In addition, HBsAg may contain several type specific antigens named a and Y and W and r as well as aothers, Gamma is predominently seen in Asia.

The third antigen associated with hepatitis B is hepatitis Bc antigen (HBcAg) which is immunologically and bio-chemically distinct from HBsAg, HBcAg and DNA polymerase.

A variety of methods for detection of HBAg and antibody to HBAg have been described. Agal gel diffusion was the first method used

by Blumberg et al (1965) which resulted in the discovery of Australia antigen. Subsequently, complement fixation test (Purcell et al 1969), electron microscopy (Shullman and Barker (1969), immune florscence (Millman et al 1970), radio-immunoassay (Walsh et al 1970), electro-osmodiffusion (Persendorfer et al 1970) and immune adherance test (Mayumi et al 1971) have been developed to detect it.

The HBAg appears late in the incubation period of hepatitis and precedes the clinical and biochemical abnormalities. The peak HBAg titer generally coincides with the on set of clinical hepatitis (Parker et al 1972), Generally in overt clinical hepatitis with complete recovery, the HBAg in serum is transient. When HBAg persists beyond 12 weeks, chronic hepatitis is common sequelae. There is abundance of literature on the relationship of Australia Antigen (HBAg) and chronic hepatic disease (Fox et al 1970, Wright, 1960, Gitnick 1969, Faber et al 1970, Velasko 1970, Dudley et al 1972. Grab et al 1972 and Sherlock 1975) chronic hepatitis which is more commonly seen in mediterranean and tropical countries. (Bianchi et al

1972), is classified into 2 types chronic persistent and chronic aggressive or chronic active hepatitis. The chronic active hepatitis is a progressive disease, resulting in cirrhosis is more than 50% cases within 5 years. Schaefor et al 1967 and Francis et al have given similar progression of the disease.

The hepatitis B antigen has been demonstrated in the liver cells in cases of chronic hepatitis and cirrhosis by immunoflorescent and/or electron-microscopy suggesting aetiological relationship (Millman et al 1969). Serial liver biopsies of antigen positive hepatitis patients have progression to chronic hepatitis, cirrhosis and in one instance to hepatoma has been reported (Sherlock et al 1970).

Recent studies with sensitive techniques have demonstrated many times higher incidence of hepatitis B antigen in hepatoma compared to normal population (Benson et al 1971). A frequency of more than 30% has been observed in Chile, Spain, Greece, India, Taiwan, Uganda, Taipeh, Senegal and Singapore (Messeyeff et al 1972., Sama et al 1972). More than 60% of hepatoma occurs in preexisting cirrhosis predominently of macronodular type (Vogal et al 1970, Sama et al 1974). This supports

the progression of acute viral hepatitis through chronic aggressive hepatitis to cirrhosis and hence malignant change in regenerating nodules.

The detection of hepatitis associated antigen in serum was simple by various methods described previously gave stable results higher to its localization in liver by immunoflorescence, electron microscopy and immunopenoxidase staining technique have been costly, time consuming and unsuitable for application in large scale studies and at newer and less equipped smaller centres. This always remained a rider on the frequency and mass studies from different zones of world.

It was in 1974 when Shikata et al demonstrated a simple, sensitive and reproducable technique for detection of hBsAg in conventional paraffin section by orcein staining. During the examination of the conventional paraffin section and comparing findings of Australia antigen demonstrated by immunoflorescente method, Shikata et al noticed that similar structures, which were demonstrated by florescent isothiocynate (FITC) labelled anti-Australia serum, were stained faintly black by elastica von Vieson stain. Further studies disclosed that this faint colour was caused by resorcin

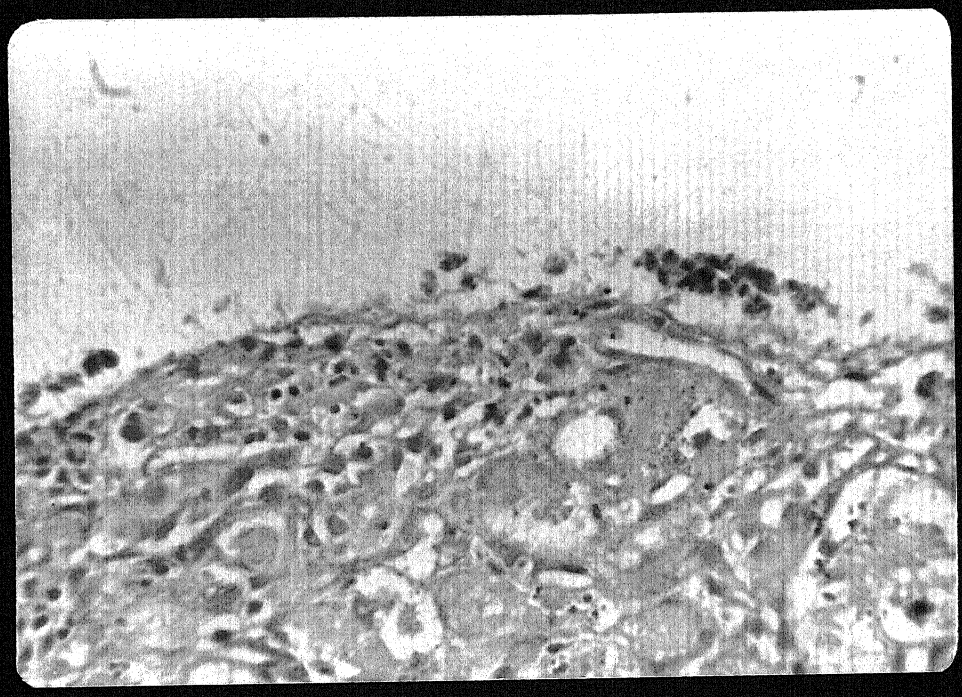
fuchsin and not by von Gieson stain. After perception of this phenomenon, it was easy way to find out better staining method for Au-antigen in paraffin sections. It was disclosed that modified orcein method demonstrated more clearly an antigen in the paraffin sections. Orcein almost selectively stained Au-antigen and was negative for other serum proteins.

The advent of this orcein technique by Shikata et al (174) revolutionised the identification of Hepatitis B surface antigen in liver tissues of chronic liver diseases, i.e., chronic active hepatitis persistent hepatitis, cirrhosis and hepatoma. Their study comprised of liver specimens of 25 autopsy case of hepatitis positive for Australia antigen. Cirrhosis liver and hepatoma and Au-carriers. They also studied 35 cases of hepatitis, liver cirrhosis, hepatoma without Australia antigenaemia as control and 20 cases with other diseases. The paraffin sections were stained by modified orcein stain and a number of other stains for comparison purposes. The workers claimed 100% positive staining results in the group of cases having Australia antigens positive hepatitis. Specific immuno-florescence and immunoperoxidases stains for HBsAg correlates well with orcein staining in the same cases and with electronmicroscopy too.

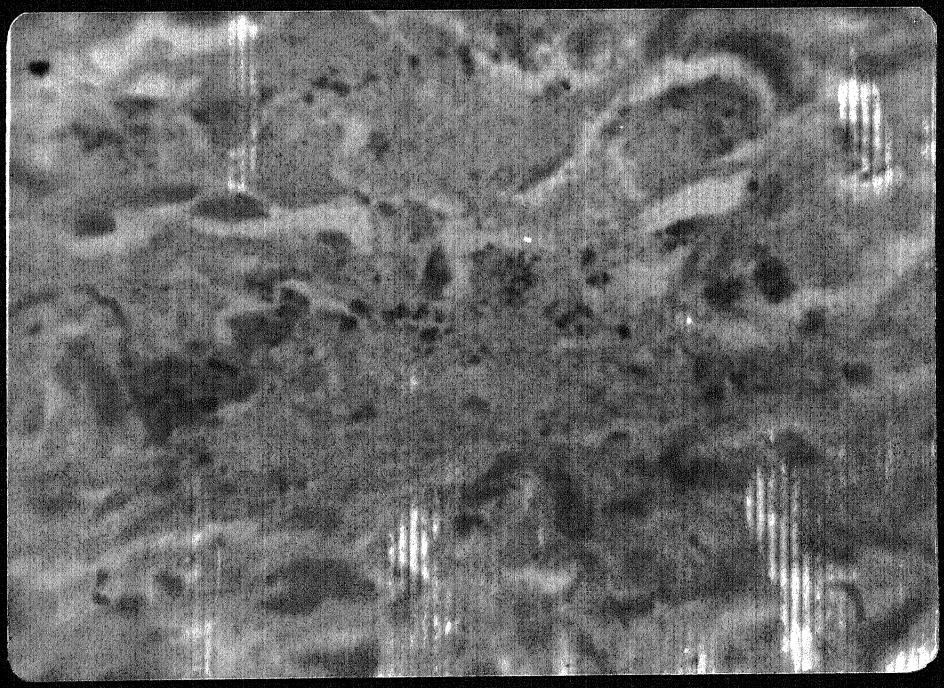
When purified Australia antigen and other serum protein were spread on Agar by electrophoresis and subsequently stained by modified orcein stain and Ponceau fuchsin, it was observed that orcein almost selectively stained Australia antigen and not the other serum proteins. (Shikata et al 1974). The Australia antigen in liver tissues stained by above methods almost completely corresponded with the case of Australia antigenaemia. The hepatocyte containing this hepatitis B surface antigen as inclusion body was later on termed as Shikata cell.

Morphology and structure of Shikata cell:

The orcein stain, which is a vegetable dye extracted from certain lichens by the action of ammonia and air, stained the antigen as amber colour which stood out prominently against the cytoplasmic background and the haematoxylin staining of the nucleus (Nayak et al 1975). The antigen is distributed very randomly in the liver, being present in a number of neighbouring hepatocytes over focal areas or in individual liver cells widely separated from each other.



Photograph showing SHIKATA CELL in LOW POWER (70 X)



Photograph showing SHIKATA CELL in HIGH POWER (280 X)

There is no predeliction of these Shikata cells to be distributed around any vascular teritories. The antigen presented essentially three distinct morphological patterns:-

(a) large, clearly outlined discrete inclusions of different shapes and sizes occupying a large portion of or occasionally the entire cytoplasm. These were occasionally seen as clumps of the inclusion bodies in the cytoplasm.

(b) Over or more round to oval vesicles and short tubules.

(c) Granules or short fine fibrils distributed along one or both sinusoidal borders or throughout the cytoplasm of the hepatocyte. This was seen as fine powder in less higher magnification.

The number of orcein positive was appreciably higher in those biopsies showing a ground glass appearance of the hepatocytes (Hadziannis et al 1973). The hepatocytes with ground glass cytoplasm in an otherwise normal liver specimen should lead

to the suspicion of a carrier state of HBAg.

The inclusion variety of the antigen could often be demonstrated in the conventional hematoxylin and eosin stain. It appeared as a very light, purplish red, relatively ill-defined, transluscent inclusions having a fine fibrillar internal structure, different from other hyaline inclusion observed in the hepatocytes. The vesicular and observed tubular forms of antigen could also be seen in the hematoxylin and eosin preparation. On the other hand, the counter part, in hematoxylin and eosin preparations of the granular and finally fibrillar form could not be appreciated.

As the incidence of the antigen is inversely proportional to the necrosis, so was not seen in necrotic areas.

The high affinity of Australia antigen for dyes used in staining techniques has been explained by the nature of antigen. The antigen possesses many disuplide bonds and Gomori's aldehyde fuchsin stains these bonds (Vyas et al 1972 a, Shikata et al, 1974), however, the mechanism of staining for orcein or resorcin stain is not clear. The antigen has been shown

to have high levels of cystine (Dressman et al 1972. Vyas et al 1972 b). It is believed that disulphide bond plays an important role in antigenicity and particle size of HBsAg (Sukena et al 1972. Vyas et al 1972 a). The more direct evidence, i.e., the antigen that is stained with orcein is in close correspondence with the positive staining and specific immunoflorescence for HBsAg (Shikata et al, 1974, Gerber et al 1974).

COMPARISON OF ORCEIN STAINING WITH OTHER TECHNIQUES

The immunoflorescene and electron microscopic studies have confirmed that ground glass hepatocytes and Shikata cells contain HBsAg (Hadziyannis et al 1973, Gerber et al 1974, Huang et al 1974). Later on Nayak et al (1975) conducted a study in which comparision of immunoflorescence, Immuno peroxidase and orcein staining method was done with regard to their specificity and reliability as antigen marker. The author observed that the specific antigen determinants of the HBsAg are not inactivated by formalin fixation and processing for paraffin embedding. The formalin treatment of the antigen does not eliminate its ability to react with specific antiserum which is also evident from the results of in vitro immunoprecipitation reactions.

Precipitation lines with both formalin treated and untreated antigen are stained well with both immunoflorescence and orcein. However, author says that working with conventionally fixed paraffin sections, interpretation of immunoflorescence section can pose greater difficulties than frozen sections of fresh tissues principally due to increased background immunoflorescence. The observation of Shikata et al (1974) that serum HBsAg can be stained by orcein and that positive immunoflorescence for antigen in frozen tissue also reveals positive orcein staining after formalin treatment of the same sections.

Nayak et al (1975) have confirmed the same by demonstrating orcein identity with both immunostaining procedures performed on same sections. Author concludes that the simplicity of orcein technique becomes more relevant for large scale retrospective and prospective studies where orcein staining offers distinct advantages. Mass screening of stored paraffin embedded liver tissues particularly obtained at autopsy will yeild important information on association of HBsAg in various forms of chronic liver diseases.

SHIKATA CELL AND CHRONIC LIVER DISORDERS

The incidence of HBAg in post necrotic and post hepatic cirrhosis in different countries show a marked variability. Faber et al 1970 reported an 18% positivity from Denmark while it was 33.2% in Italy as reported by Carrella (1972) by Agar gel diffusion and complement fixation techniques respectively. The incidence of HBAg in cirrhosis of cryptogenic and mixed variety varied from 2% in Great Britain by Fox et al 1969 to 59.2% in India by Sama et al using Agar gel diffusion and Radio-immunoassy technique respectively. A number of other workers have demonstrated the presence of HBsAg in hepatocytes in chronic hepatic diseases (Hadziyannis et al 1972, Gerber et al 1974 and Poper 1975).

Deodher et al (1975) in their study used modified orcein staining technique. The method differed slightly from the original one. The oxidising solution used by them consisted of 0.15 gm of KMn_4, 100 ml distilled water and 0.015 ml. conc. sulphuric acid, instead of 0.3% $KMno_4$ solution. The sections were decolourized in 2% oxalic acid for 10 minutes. The differentiating medium was 1% acid alcohol

instead of absolute alcohol. In their study of 97 seropositive patients, 33 consisted of acute hepatitis which came out all orcein negative. This reflects a genuine absence of the antigen as observed by Dudley et al (1971). In the rest 64, comprising of chronic persistent hepatitis, chronic active hepatitis, cirrhosis, liver cell carcinoma and HbsAg carries there were 41 orcein positive cases.

The author contributed their discrepancy with the result of Shikata et al (1974) to the sampling error, as there exists a vide variability of orcein positive cell within the same biopsy.

Seth et al (1975) studied the incidence in adult cirrhotics and came up with a positivity of 37% in needle biopsy cases while it was 80% in autopsy cases and 78% in wedge biopsy cases.

Nayak et al (1975) demonstrated the HBsAg in paraffin sections of cirrhosis and primary hepatocelluar carcinoma by three different methods viz., immunoflorescence, Immunoperoxidase and orcein. The HBsAg positivity in cirrhosis by above techniques 42.8%, 60% and 70% respectively.

The figure for hepatocellular carcinoma were 75%, 90% and 100% respectively. The localization was in fact by all these three methods, but positivity was higher and detection simple by the latter two methods.

Later on Kostich et al (1977) came with a 55% positivity in liver biopsies from patient of chronic hepatitis using the same technique. The author claimed that this method is useful not only in diagnosing the aetiology of chronic from acute hepatic diseases but also establishing the aetiology of cirrhosis in patients and stored autopsy materials.

Aikat et al (1977) included 230 liver tissues, 115 from autopsy and 115 from biopsy material and demonstrated Shikata cell in 50% cases of macronodular and 52% of mixed noduler cirrhosis. In the hepatoma with cirrhosis, Shikata cells were seen in regenerating nodules of 79% cases and in none of the cases of hepatoma without cirrhosis in autopsy group. The incidence in biopsy groups was 23% in macronoduler cirrhosis and 19% in a chronic active and chronic persistent hepatitis. The significant difference observed in autopsy and biopsy material can be directly corelated with the amount of hepatic tissue examined.

In the year 1979 a positivity of 32.6% was revealed by Priyadarsini et al after a study of 187 biopsies including all the chronic liver diseases. The cirrhosis group comprised 36.1% and 66.7% in needle & wedge biopsy respectively while the % for hepatoma with cirrhosis was 66.7% and 100% respectively. The hepatitis with cirrhosis group showed % of 16.7% and 50% respectively in needle & wedge biopsy.

However, Ho et al (1980) in a study of 70 cases of cirrhosis and hepatocellular carcinoma using Gomori aldehyde fuchsin stain, reported 90% correlation between tissue and sera positivity for HBsAg. The positivity was 68% histochemically for HBsAg and 71% serologically in cirrhotics.

Recently Kudo et al (1982) conducted a study where a comparative study of histopathology and frequencies of hepatitis B marker was done. He reported 36.5% and 27.8% in two groups of cirrhosis classified on basis of severity. The instance of HBsAg positivity was only 10% in non-cirrhotics and 31.6% in hepatocellular carcinoma. This positivity was almost identical though less in comparision to serum positivity of HBsAg.

While utilising the orcein staining technique

Masachika seneba (1982) noticed that the degenerative and necrotic cytoplasm of hepatocytes was too stained and created difficulty in identification of HbsAg. To get still better and crystal clear view of the HBsAg he proposed a modification in the usual orcein technique by utilising a senstiser solution, i.e. 2% Fernic ammonium sulphate solution or 10% uranium nitrate solution. This sensitisor solution filled the gaps of the protein with iron and left no room for orcein dye and so where the tissue was kept in orcein, the cytoplasm was not stained. This gave stable and satisfactory results.

A study by Chadda et al (1982) of 120 cases of various liver disorders from Rajasthan showed total positivity of 16.7%. On further categorising the group it was 22.0% in cirrhotics, 12.5% in chronic active hepatitis and 4.8% in hapatoma. The different studies in India show variation in percentage of orcein positivity which clearly supports the fact that there exists a geographical variation of the disease as regard to its incidence (Seth et al 1975).

Shikata cell and Indian Childhood cirrhosis

Indian childhood cirrhosis is such a disease whose clinical and histological profile is well

known but clouds still cover its exact astiology, which still remains a mystery. The probable factors proposed for it include infective hepatitis virus, auto immunity, defect in carbohydrate metabolism, toxic agents like aflatoxin and genetic factors.

Chandra (1970) postulated that the defective celluler immunity makes the liver more vulnerable to the hepatitis virus. He also showed presence of Hepatitis associated antigen in 20% of his cases of I.C.C. In the study conducted by Dhatt et al of 27 cases 6 were of infective hepatitis. One of which later on proved to be positive for HAA by modified double diffusion micro-ouchterlony technique using 1% agar gel. The author attributed that some of the cases clinically presenting as infective hepatitis, may in fact be serum hepatitis cases in whom virus has been transmitted by oral route. However, recently Chadda et al (1982) confirmed the findings of Chandra (1970) and reported a higher incidence of 40% positivity in their study of 35 patients of Indian childhood cirrhosis though the serum of five patients, who showed orcein positive grannules in the hepatocytes, was negative for HBsAg and anti HB. The

author concludes that this orcein positive granules in the I.C.C. represent non-HBsAg copper binding protein due to rich sulphryl group has affinity for orcein staining.

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MATERIAL & METHOD

MATERIAL AND METHODS:

The present study was conducted in all the suspected cases of hepatic cirrhosis admitted in indoor Medical and Paediatrics Wards of Maharani Laxmi Bai Medical College & Hospital, Jhansi, during the period of July, 1982 to March, 1983. All the cases were followed up during this period of study for any further complications.

A detailed and relevant clinical history of each patient was recorded. Emphasis was laid on the past history of any attack of jaundice (viral hepatitis), blood transfusion, intravenous and intramuscular injections, immunisations, gamma globulin injections, surgical operations and anaesthesia etc. A detailed history of drug intake, like methyl dopa, methotrexate, oxyphenacetin, oral contraceptive, anaesthetic agents, intravenous tetracyline, isonex, para amino salicylic acid and rifampicin etc. was noted.

A special note was taken of the dietory history of patient to assess the daily protein intake. Regarding personal history, the alcohol intake, its frequency, amount and duration was noted in detail. Diseases like

prolonged cholestasis, storage diseases, Cardiac failure, haemachromatosis, Syphilis and Wilson's disease, were excluded carefully in each case.

Suspected cirrhosis patients were subjected to a questionnaire and the observations were charted in the case sheet (Annexure-1).

Relevant laboratory investigations were performed in all the cases which included blood examinations - Total leucocyte count, Differential leucocyte count, Erythrocyte sedimentation rate and Haemoglobulin percentage.

The bleeding time was performed by Finger prick technique while coagulation time was done by Dale and Laid-low technique. The platelet count was done by visual method using improved Neubaur's chamber & formal citrate as diluent. The prothrombin time was done by Quick's one stage technique to rule out bleeding tendencies.

The urine examination was done for presence of bile salts by Hay's Test, while bile pigments and urobilinogen by Fouchet's test and Wallace and Diamonds quantitative test respectively. Before proceeding for liver biopsy the liver functions were assessed by a battery of liver

functions tests. These included vanderbergh test performed by method of Malloy & Evelyn (1937) technique, serum bilirubin estimation by modification of Malloy Evelin's method and thymol turbidity test (Maclagan, 1944 b). Total serum, protien, albumin and globulin were estimated by Biuret method. (Mein hold (1953)).

All the three enzymes, serum glutamic oxaloacetic transaminases, pyruvic transaminases and serum alkaline phosphotases were estimated by colorimetric method (Rietman and Frankel 1957).

Prior to the liver biopsy each patient was given an intramuscular injection of 10 mg. vitamin K. daily for three days. The liver biopsy was performed via intercostal space using a Vim-Silverman Needle. In a few cirrhotic patients admitted for abdominal surgery, the liver biopsy was performed during laprotomy.

The biopsy tissue was fixed in 10% formalin (100 ml 40% formaldehyde in 900 ml of distilled or tap water). The tissue was later on taken to histo-pathology section of Pathology Department of Maharani Laxmi Bai Medical College, Jhansi, for tissue processing.

Tissue processing: The tissues were loaded in tissue catchets and put in automatic tissue processing machine

(YORCO type) and passed through grades of alcohol, then through chloroform. The tissues were ready for wax blocking on the next day. The processed tissue was immersed in a wax filled trough made by Leuckhart's 'L' pieces and later on duly labelled. On solidification of the wax, the mould was removed and a block holder was attached to the surface of the block which was opposite to the tissue. The surface towards the mould's base was trimmed.

The paraffin section 5μ thickness were cut by senior Rotatory microtome and 2-3 sections were transferred on two albumen coated slides after a ribbon of sections were floated on warm water. The sections were then dried on a hot plate at 60-70° C for 10 minutes after which one section was stained by haematoxylin and eosin and other with orcein stain.

STAINING OF SLIDES

(A) Haematoxylin and Eosin staining.

Preparation of Stain

1. Haematoxylon stain (Haris 1900) 2.5 grams of haematoxylin was dissolved in 25 ml. absolute alcohol and to it was mixed a solution of 50 grams of potassium alum dissolved in 500 ml. of warm distilled water. This was then rapidly cooled down by plunging flask in cold water and 20 ml. of glacial acetic acid was added

to it for better results. 1.25 gram of mercuric oxide was added as preservative of stain.

2. Eosin Stain 1% solution was made indistilled water and 0.5 ml. of glacial acetic acid was added in a litre of stain for sharpness of stain. A crystal of thymol was added as preservative to inhibit fungal growth.

3. Acid Alcohol 1% 70 ml. absolute alcohol was mixed with 30 ml. water and to it 1 ml. conc. hydrochloric acid was added.

Staining technique of Harris Haematoxylin and Eosin stain:-

1. The dried and cooled slides were dipped in Xylene for 2-5 minutes to dissolve the wax.

2. The dewaxed sections were then hydrated to water after passing via the following mentioned concentration of alcohol:-

(i) 100% alcohol 2 min.
(ii) 90% alcohol 2 min.
(iii) 80% alcohol 2 min.
(iv) 70% alcohol 2 min.
(v) 50% alcohol 2 min.

then in water for 5 minutes.

3. The slide was then dipped in Harris Haematoxylin stain for 5 minutes.

4. The sections were washed in tap water until section turned 'blue'.

5. Differentiated in 1% acid alcohol for 5-10 seconds.

6. Then stained in 1% Eosin stain for 1 minute and washed well in tap water.

7. The tissues were then dehydrated by passing in following mentioned grades of alcohol:-

(i) 50% alcohol 2 min.
(ii) 70% alcohol 2 min.
(iii) 80% alcohol 2 min.
(iv) 90% alcohol 2 min.
(v) 100% alcohol 2 min.

8. The tissues were cleared by dipping in Xylene for 2 min.

9. Then tissues were mounted in D.P.X. (Disterene 80 - 10 gm., Dibutylphthalate 5 ml. xylene 35 ml.)

Results: (1) the nuclei - blue stain
(2) Cytoplasm - pink stain.

The slides which showed evidence of early cirrhosis,

cirrhosis, chronic active hepatitis or hepatocellular carcinoma were then stained by orcein stain.

ORCEIN STAINING:

In this study the orcein technique as modified by Masachika seneba was adopted.

Preparation of Stain:

1) Orcein stain - One gram of Orcein dye (Loba) was dissolved in 100 ml. of 70% alcohol, whose pH was previously adjusted to 1-2 by adding 1.2 ml of concentrated Hcl. The supernatent available after 2-3 days was utilised to stain the slides.

2) Oxidising solution: 300 mg. of potassium permanganate was dissolved in 100 ml. of distilled water to which 0.3 ml. of sulphuric acid was added.

3) Decolourising solution: 1.5 gms. of oxalic acid was dissolved in 100 ml. of distilled water at room temperature.

4) Sensitiser solution: A solution of 2% Ferric Ammonium Sulphate was prepared in distilled water.

5) Haematoxylin solution: (Harris 1900) as described previously in the haematoxylin and eosin staining technique.

6) Acid Alcohol: To 100 ml. absolute alcohol,

1 ml. conc. Hydrochloric acid was added.

Masachika seneba's Modified staining technique

1. The sections were deparaffinised by keeping them in xylene for 2 min.

2. Hydrated to water through the following steps:-

(a) Dipped in 100% alcohol - 2 min.
(b) 90% alcohol - 2 min.
(c) 80% alcohol - 2 min.
(d) 70% alcohol - 2 min.
(e) 50% alcohol - 2 min.
(f) Kept under running water for - 2 min.

3. Dipped in oxidising solution for 5 min.

4. Washed in running water for 5 min.

5. Treated with decolourising solution until decolourised.

6. Washed in running water for 2 min.

7. Treated with sensitiser solution.

8. Washed in running water for 2 min.

9. Treated with orcein solution for 5-10 min.

10. Dipped in absolute alcohol.

11. Differentiated in acid alcohol for 5 min.

12. Washed in running water for 5 min.

13. Stained in haematoxylin solution for 2 min.

14. Washed in running water for 5 min.

15. Dehydrated by following procedures:-
    a) 50% alcohol for 2 min.
    b) 70% alcohol for 2 min.
    c) 80% alcohol for 2 min.
    d) 90% alcohol for 2 min.

16. Cleared in xylene for 1 min.

17. Mounted in DPX.

Interpretation

(1) HBsAg, elastic fibres and bile pigments are stained brown.
(2) Erythrocytes, mast cells, degenerative and necrotic cytoplasm are not stained.
(3) The nucleus is stained blue.

The HBsAg was of variable shaped, i.e., powdery, granuler, clumps or as fine fibrils. The hepatocytes containing HBsAg is called as Shikata cell. The

possibility of HBsAg is inversely proportional to the necrosis and so it is not seen in necrotic areas. The maximum of it is seen in ground glass hepatocytes, existing in the peripheral region of cytoplasm near the sinusoids. The positive material can also be found in Kupffer cells. If the ground glass hepatocyte is seen then, the specimen will also have Shikata cells.

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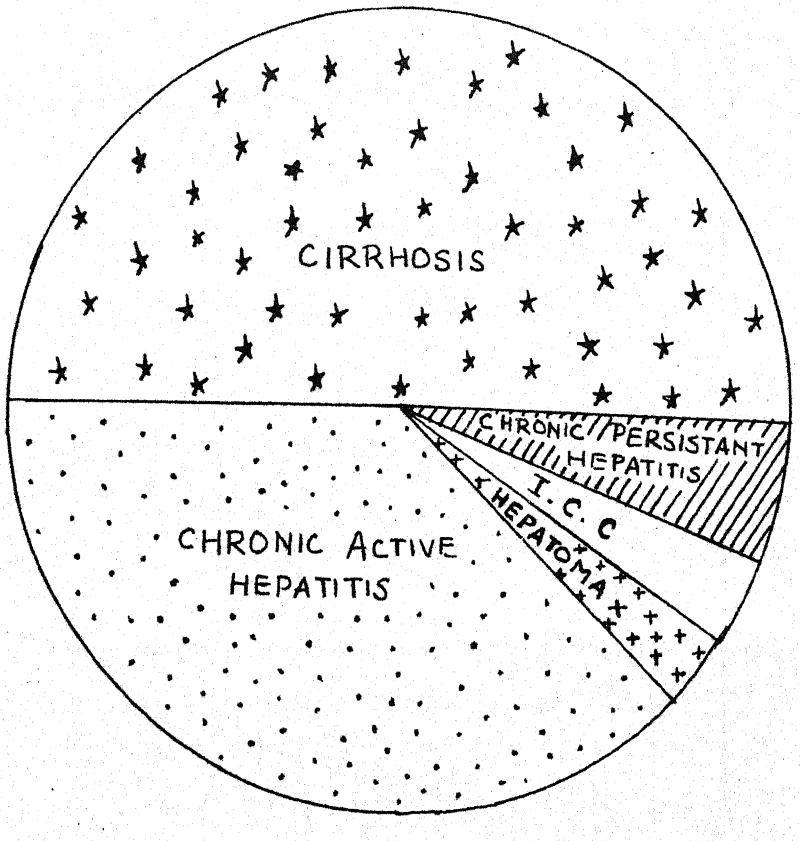
OBSERVATION



PIE DIAGRAM SHOWING DISTRIBUTION OF DIFFERENT CHRONIC LIVER DISEASE

OBSERVATION:

The present study was conducted at M.L.B. Medical College & Hospital, Jhansi, which comprised of 97 cases of chronic liver diseases viz., cirrhosis, chronic active hepatitis, chronic persistant hepatitis, hepatoma and Indian childhood cirrhosis.

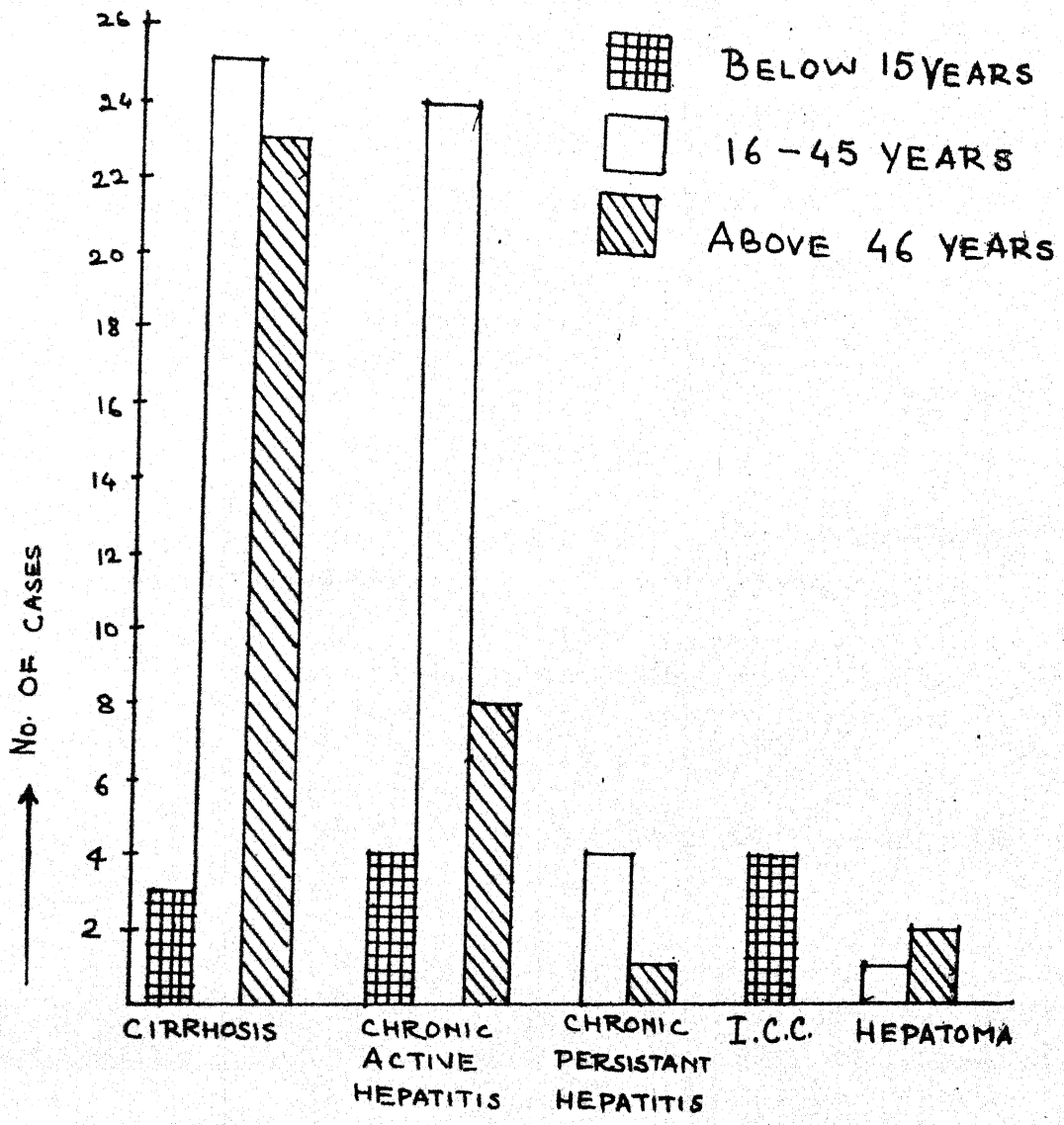
Out of these 97 cases, the 77 liver biopsies were taken from the patients admitted in M.L.B. Medical College Hospital during a period of one year of the study while rest 20 biopsies were studied from the stored liver tissues of chronic liver diseases from the Department of Pathology.

The different sub-groups of chronic liver diseases which were studied are depicted in Table I which shows number of subjects in different groups:

TABLE I: Number of subjects in different groups

Sub-Groups	Male	Female	Total
CIRRHOSIS	31	18	49
CHRONIC ACTIVE HEPATITIS	21	15	36
CHRONIC PERSISTANT HEPATITIS	1	4	5
INDIAN CHILDHOOD CIRRHOSIS	4	0	4
HEPATOMA	1	2	3
TOTAL	58	39	97

The maximum number of cases 49 (50.5%) belonged



BAR DIAGRAM SHOWING AGE DISTRIBUTION OF DIFFERENT CHRONIC LIVER DISEASES

to cirrhosis while minimum number of case 3 (3.08%) were of hepatoma.

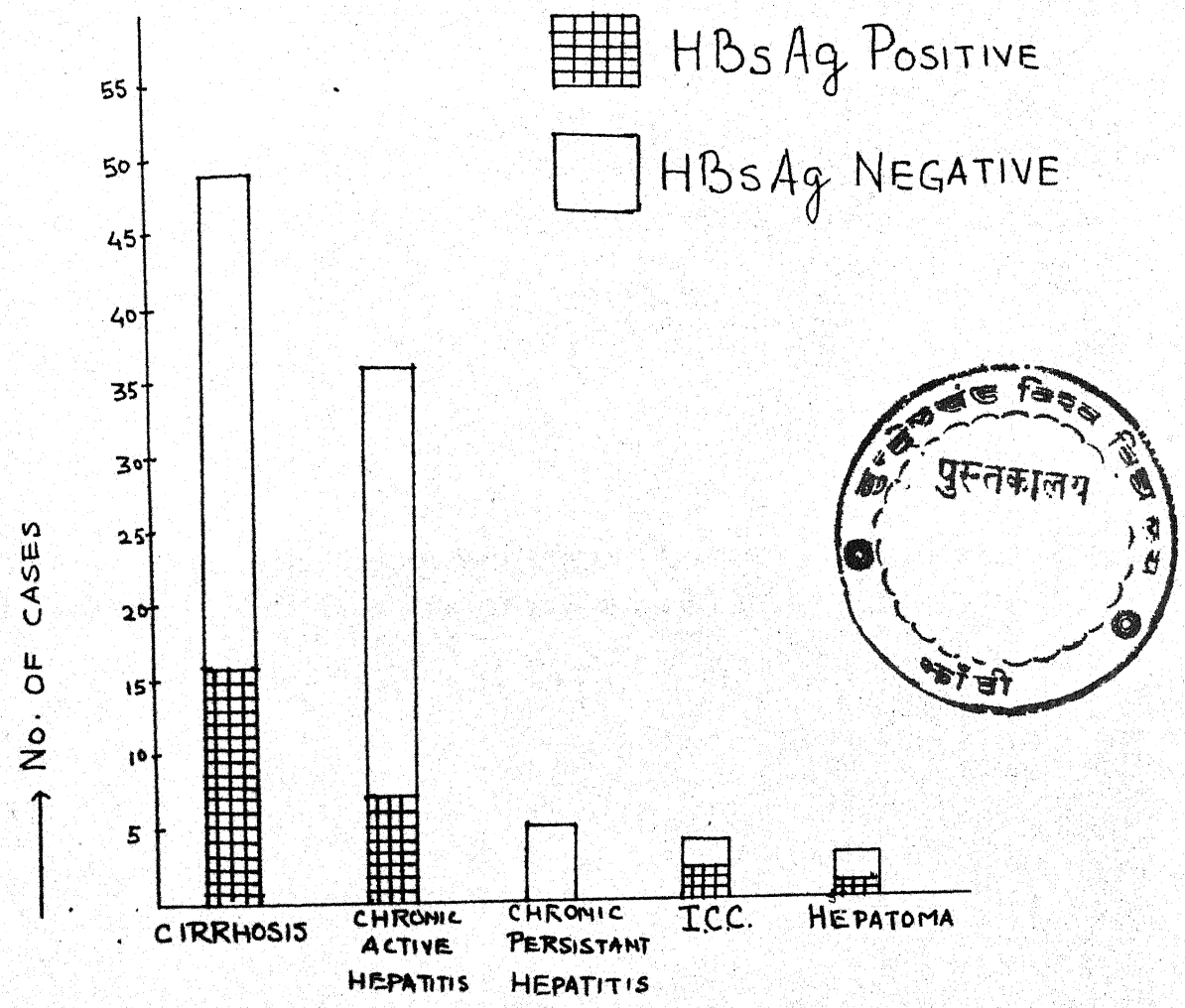
Amongst the 49 cases of cirrhosis sub-group males were 31 (63.2%) while females were 18 (36.8%). Out of the total of 36 cases diagnosed as chronic active hepatitis, males were 21 (58.3%), while females were 15 (41.7%). The number of chronic persistant hepatitis, Indian childhood cirrhosis and hepatoma in total were 12 (12.3%) only and in all sub-groups male dominated the number.

The Table II shows the age distribution of different sub-groups of subjects:

TABLE II: Age distribution of different sub-groups of subjects.

Age Distribution	Sub Groups					
	Cirrhosis	Chronic active Hepatitis	Chronic persistant Hepatitis	Indian childhood cirrhosis	Hepatoma	TOTAL
Below 15 years	3	4	0	4	0	11
Between 16-45 years	25	24	4	0	1	54
Above 46 years	21	8	1	0	2	32
TOTAL	49	36	5	4	3	97

The maximum number of cases 54 (55.5%) belonged to



COMPONENT BAR DIAGRAM SHOWING SHIKATA CELL POSITIVITY IN DIFFERENT CHRONIC LIVER DISEASES

age group ranging from 16 years to 45 years, while minimum number of cases were below 15 years i.e., 11 (11.3%) only.

TABLE III: Shikata cell positivity in different sub-groups of chronic liver diseases

Variable	Total no. of cases	Total no. of positive	Percentage of positivity
1. Cirrhosis	49	16	32.69%
2. Ch. Active Hepatitis	36	7	19.44%
3. Ch. Persistant Hepatitis	5	0	0%
4. Indian Childhood Cirrhosis	4	2	50%
5. Hepatoma	3	1	33.33%
GRAND TOTAL	97	26	27.83%

SHIKATA CELLS IN DIFFERENT SUB-GROUPS

In 49 cases of cirrhosis (40 fresh & 9 stored) the orcein staining showed presence of Shikata cell in total of 16 cases (32.69%), while it was absent in 33 cases (67.31%). Shikata cells were positive in 7 (19.44%) cases of chronic active hepatitis, while in chronic persistant hepatitis, the positive staining

was nil. In the Indian childhood cirrhosis sub-group 2 (50%) cases were positive while it was seen in only 1 (33.3%) cases of hepatoma.

By this observation it is evident that maximum positivity of Shikata cell was present in cases of Indian childhood cirrhosis (50%), Hepatoma (33%) and Cirrhosis (32.69%), while no case was found positive for Shikata cell in Chronic persistant hepatitis and only 19.44% positive cases in Chronic active hepatitis sub-group. The overall positivity of Shikata cell was observed in 26 (27.83%) cases.

SHIKATA CELL IN RELATION OF CLINICAL PROFILE OF DIFFERENT SUB-GROUP:

(A) CIRRHOSIS: The table IV is showing the relationship of various symptoms of cirrhosis and positivity of Shikata cells.

In the Shikta cell positive group 7 (50%) cases gave past history of jaundice, while it was absent in remaining 7 (50%) cases. In the Shikata cell negative group the history of jaundice was present in 18 (69.7%) cases while it was absent in 8 (30.7%) cases. The observation indicating that the presence

TABLE IV

SHIKATA CELLS IN RELATION OF VARIOUS SYMPTOMS IN CIRRHOSIS CASES.

SYMPTOMS	Shikata cell positive		Shikata cell negative	
	No.	Percentage	No.	Percentage
1. Jaundice				
(A) Present	7	50	18	69.3
(B) Absent	7	50	8	30.7
2. Peritoneal tapping				
(A) Present	9	64.3	13	50
(b) Absent	5	35.7	13	50
3. Odema feet				
(A) Present	9	64.3	17	65.3
(B) Absent	5	35.7	9	34.7
4. Loss of libido				
(A) Present	8	57.1	14	53.8
(B) Absent	6	42.9	12	46.2
5. Immunisation				
(A) Present	10	71.4	25	96.2
(B) Absent	4	28.6	1	3.8
6. Haematemesis or malena				
(A) Present	5	35.7	9	34.7
(B) Absent	9	64.3	17	65.3

or absence of history of jaundice has no bearing regarding the positivity or negativity of Shikata cell. (X^2= ·731, d.f. = 1, p <0.5).

The history of peritoneal tapping was affirmative in 9 (64.3%) cases of HBsAg positive group and negative in 5(35.7%), while the same history in HBsAg negative group was present in 13 (50%) and negative in rest 13(50%) cases. This suggests that a history of peritoneal tapping i.e., evidence of ascitis or its absence in a case of cirrhosis also does not have any influence upon the positivity of Shikata cell. This finding is statistically insignificant (X^2= ·983, d.f.=1, p <0·5).

Out of the 14 HBsAg positive cases 9(64.3%) had past history of pedal odema and 5 (35.7%) had none. The values in HBsAg negative group were 17 (65.3%) and 9 (34.7%) respectively. As these values are statistically insignificant (X^2= ·065, d.f.= 1,p <0.8) which is an indication that presence or absence of pedal oedema in a cirrhosis case does not support or rule out the possibility of its HBsAg positivity.

In the patients who showed presence of Shikata cells the loss of libido was present in 8 (57.1%) cases only while it was not altered in 6 (42.9%). In the negative group the loss of libido was reported by 14 (53.8%) only and was normal in 12 (46.2%). These values are statistically insignificant ($X^2 = .282$ d.f.=1, $p < .7$) hence it indicates that loss of libido has no relation with Shikata cell positivity.

A past history of immunisation or parentral therapy was present in 10 (71.4%) cases of HBsAg positive group and was absent in 4 (28.6%) but it was present in 25 (96.2%) and absent in 1 (3.8%) of HBsAg negative group. These data reflects that positive history of immunisation or parentral therapy in past has no influence regarding the positivity of Shikata cell in cirrhosis. As 4 of Shikata cell positive group denied any past history of parentral therapy while it was only one in Shikata Cell negative group, (hence there is some definite other non-parentral route of infection of serum hepatitis which infected this group). These values are shatistically significant ($X^2 = 6.013$ d.f.=1 $P < .02$).

A history of gastro-intestinal haemorrhage, i.e.,

haematemesis or melena was present almost equally in both groups i.e., 5(35.7%) of the HBsAg positive and 9(34.7%) of HBsAg negative group. The history was negative in rest of 9 (64.3%) and 17 (65.3%) cases respectively. These values are statistically not significant ($X^2 = .138$, d.f. = 1 $p > 0.7$) and indicate that gastro-intestinal haemorrhage may not be the deciding symptom of Shikata cell positivity or negativity in a cirrhosis case.

Thus it is reasonably clear that the Shikata cell positivity or negativity cannot be predicted on the basis of symptoms of cirrhosis.

The relationship of various signs of cirrhosis cases with Shikata cell positivity is depicted in Table V.

The jaundice was equally prevelant in both groups, i.e., present in 4 (28.6%) of the HBsAg positive cirrhosis cases while it was absent in 10 (71.4%). It was present in 7 (26.9%) cases of HBsAg negative cirrhosis and absent in 19 '73.1%) cases. As these values are statistically not significant ($X^2 = .173$, d.f. = 1, $p > 0.5$) hence it can be inferred from above table that presence of jaundice or its absence has no bearing regarding the positivity of Shikata cell.

Anaemia was noted in 13 (92.8%) cases of HBsAg positive group and absent in 1 (7.2%) only while it was present in 19 (79.1%) cases of HBsAg negative group and absent in 5 (20.9%). These values are statistically insignificant ($\chi^2 = 1.161$, d.f.= 1, $p > 0.2$) hence it indicates that presence of anaemia or its absence may not help to decide the Shikata cell positivity, clinically.

TABLE V

SHIKATA CELLS IN RELATION OF VARIOUS SIGNS OF CIRRHOSIS

Signs.		HBsAg positive Number	%	HBsAg Negative Number	%
1. Jaundice	(A) Present	4	28.6	7	26.9
	(B) Absent	10	71.4	19	73.1
2. Anaemia	(A) Present	13	92.8	19	79.1
	(B) Absent	1	7.2	5	20.9
3. Collateral Circulation.	(A) Present	6	42.9	9	34.7
	(B) Absent	8	57.1	17	65.3
4. Ascitis	(A) Present	9	64.3	13	50.0
	(B) Absent	5	35.7	13	50.0
5. Splenomegoly	(A) Present	9	64.3	15	57.6
	(B) Absent	5	35.7	11	42.4
6. Hepatomegoly	(A) Present	2	14.2	13	50.0
	(B) Absent	12	85.8	13	50.0
7. Evidence of Hepaticencephalopathy.	(A) Present	6	42.9	9	34.7
	(B) Absent	8	57.1	17	65.3

Out of the 14 cirrhosis cases who showed positive orcein staining 6 (42.9%) had evidence of collateral circulation while rest 8 (57.1%) cases had none. In the negative orcein, stainin group 9 (34.7%) had its evidence while 17 (65.3%) had no evidence of collateral circulation. These values are too statistically insignificant ($\chi^2 = .371$, d.f. = 1, $p > 0.5$) hence evidence of collateral circulation do not have any bearing regarding po itive or negative orcein staining.

An evidence of fluid in the abdominal cavity was seen in 9 (64.3%) of the Shikata cell positive cirrhotics, while it was absent in rest 5 (35.7%). Ascitis was present in 13 (50.0%) of the Shikata cell negative cirrhotics while it was absent in rest half. The values statistically insignificant. ($\chi^2 = .983$, d.f. = 1 $p < 0.5$). These data indicate that presence of ascitis has no bearing regarding the presence or absence of Shikata cell.

Splenomegaly, an evidence of postal hypertension was noted in 9 (64.3%) of the HBsAg positive group while, it was not enlarged in 5 (35.7%) only. The value for HBsAg negative group are 15 (57.6%) and 11(42.4%) respectively. These findings are in accordance that Shikata cell positivity has no bearing regarding the

presence or absence of splenomegaly. The values are statistically insignificant. ($X^2 = .553$, d.f. = 1, $p < 0.5$).

Evidence of hepatomegaly was seen in 2 (14.2%) the HBsAg positive and it was not palpable in rest 12 (85.8%) cases which the same values in HBsAg negative group were 13 (50.0%) and 13 (50.0%) respectively. Although these data indicate that liver is not palpable in majority of the HBsAg positive cases but as the results are statistically insignificant ($X^2 = .534$, d.f. = 1 $p < 0.5$) so this cannot be taken as a criteria to label a case HBsAg positive.

The evidence of hepatic encephalopathy was seen in 6 (42.9%) cases was absent in 8 (57.1%) cases of the Shikata cell positive group. While the values for Shikata cell negative group were 9 (34.7%) and 17 65.3%) respectively. A discrepancy is apparently noted by these values in the two groups but as the values are statistically not significant ($X^2 = .138$, d.f. = 1, $p > 0.7$) hence it cannot be taken as criteria to label Shikata cell positive or negative.

Thus it is definite that a cirrhosis case cannot be categorised Shikata cell positive or negative on clinical grounds.

(B) CHRONIC ACTIVE HEPATITIS:

The table VI shows the relationship of various symptoms of chronic active hepatitis and positivity of Shikata cells.

TABLE VI

SHIKATA CELLS IN RELATION OF VARIOUS SYMPTOMS IN CHRONIC ACTIVE HEPATITIS CASES.

SYMPTOMS		HBsAg Positive No.	HBsAg Positive Percentage	HBsAg negative No.	HBsAg negative Percentage
1. Jaundice	(A) Present	4	66.7	9	37.5
	(B) Absent	2	33.3	15	62.5
2. Peritoneal tapping	(A) Present	4	66.7	17	70.8
	(B) Absent	2	33.3	7	29.2
3. Swelling over feet	(A) Present	4	66.7	17	70.8
	(B) Absent	2	33.3	7	29.2
4. Loss of Libida	(A) Present	5	83.3	13	54.6
	(B) Absent	1	16.7	8	32.9
				3 N.A.	12.5
5. Immunisation	(A) Present	5	83.3	20	83.3
	(B) Absent	1	16.7	4	16.7
6. Haematemesis or Malena	(A) Present	2	33.3	2	8.4
	(B) Absent	4	66.7	22	91.6

In the Shikata cell positive group of chronic active hepatits, 4 (66.7%) cases gave a past history of jaundice, while it was absent in rest 2 (33.3%) only. While in the Shikata cell negative group, 9 (37.5%) cases gave such history while 15 (62.5%) lacked it. The above values indicate apperently that a history of jaundice may increase the probability of its positivity regarding Shikata cell, but the values are statistically insignificant ($\chi^2 = 2.082$, d.f. = 1, $p > .1$).

Out of the 6 of HBsAg positive chronic active hepatitis cases, 4 (66.7%) cases gave a past history of peritoneal tapping but negatived by rest 2 (33.3%). The same values in HBsAg negative group were 17 (70.8%) and 7 (29.2%) respectively. As the values are statistically insignificant ($\chi^2 = 0.062$, d.f. = 1 $p > .8$). So it indicates that a history of peritoneal tapping has no bearing regarding the presence of Shikata cell.

Similar values were noted in case of a history pedal oedema hence it also do not have any influence regarding orcein positive staining in a case of chronic active hepatitis.

A history of loss of libido was given by 5 (83.3%) of the Shikata cell positive group and it was normal in 1 (16.7%) only while the values for Shikata cell negative group were 13(54.6%) and 8 (32.9%) respectively. In 3(12.5%) case history of loss of libido was not applicable as they had not yet attained puberity. These values are statistically highly significant (X^2= 5.125, d.f.=1, $p > 0.02$) which indicate that loss of libido is found in higher percentage of Shikata Cell positive of chronic active hepatitis.

Out of the 6 HBsAg positive, 5 (83.3%) gave a history of immunisation or perentral therapy while it was absent in 1 (16.7%) only. Similar values were seen in HBsAg negative group. As the values are statistically insignificant (X^2= .375, d.f.=1, $p > 0.5$) it indicates that it is a chance finding that it is equal in both groups and its presence has no bearing regarding positivity of HBsAg.

A history of haematemesis or melena was seen in 2 (33.3%) of the 6 chronic active hepatitis sub-group who showed positive orcein staining while it was absent in rest 4 (66.7%) cases. The value of negative orcein staining group were 2 (8.4%) and 22 (91.6%) respectively. The values are statistically significant ($X^2 = 4.244$, d.f.$=1$, $p < .05$). This indicate that as apparent by this data that a history of haematemesis or melena is found in higher percentage of HBsAg positive group than in HBsAg negative group and may help in clinically categorising a case of chronic active hepatitis.

The table VII depicts the relationship of various signs of chronic active hepatitis and Shikata cell positivity.

Jaundice was present in 5 (83.3%) cases of chronic active hepatitis who showed Shikata cell positivity while it was absent in 1 (16.7%) case only. It was seen only in 2(8.3%) case of the Shikata cell negative group and absent in 22 (91.7%) cases. These values are statistically highly significant ($X^2 = 16.203$, d.f. $= 1$, $p < .001$) which indicate that presence of jaundice in a case of chronic active hepatitis has definitely a bearing regarding the positivity of Shikata cell.

TABLE VII

SHIKATA CELL IN RELATION OF VARIOUS SIGNS OF CHRONIC ACTIVE HEPATITIS.

Symptoms		HBsAg Positive		HBsAg Negative	
		No.	%	No.	%
1. Jaundice	(A) Present	5	83.3	2	8.3
	(B) Absent	1	16.7	22	91.7
2. Anaemia	(A) Present	5	83.3	21	87.5
	(B) Absent	1	16.7	2	12.5
3. Collateral Circulation	(A) Present	1	16.7	9	37.5
	(B) Absent	5	83.3	15	62.5
4. Ascitis	(A) Present	4	66.7	17	70.8
	(B) Absent	2	33.3	7	29.2
5. Splenomegaly	(A) Present	4	66.7	15	62.5
	(B) Absent	2	33.7	9	37.5
6. Hepatomegaly	(A) Present	3	50.0	11	45.8
	(B) Absent	3	50.0	13	54.2
7. Evidence of Hepaticencephalopathy.	(A) Present	1	16.7	9	37.5
	(B) Absent	5	83.3	15	62.5

Out of the 6 cases of HBsAg positive group, anaemia was present in 5 (83.3%) and absent in 1 (16.7%) case only. The similar values for HBsAg negative group were 21 (87.5%) and 3 (12.5%) respectively. The values are statistically insignificant ($\chi^2 = 1.622$, d.f. = 1, $p > 0.2$). These indicate that presence of anaemia has no bearing regarding the positivity of Shikata cell.

Signs of collateral circulation were noted in only 1 (16.7%) case of orcein positive group and was absent in rest 5 (83.3%) Collateral circulation was seen in 9 (37.5%) of the orcein negative group and absent in 15 (62.5%). This indicates that there is no correlation regarding the collatsal circulator and orcein positivity. The results are statistically insignificant ($\chi^2 = .44$, d.f. = 1, $p > 0.5$).

Ascitis was present in 4 (66.7% of the 6 cases who showed presence of Shikata cell and absent in 2 (33.3%) only. It was present in 17 (70.8%) of the HBsAg negative and absent in 7 (29.2%) only. These data indicate that presence or absence of ascitis in a case of chronic active hepatitis do not have any bearing regarding its Shikata cell positivity as the results are statistically insignificant ($\chi^2 = .062$, d.f. = 1, $p > 0.8$).

Splenomegaly, a sign of portal hypertension was present in 4 (66.7%) of the 6 cases who showed presence of Shikata cell, while it was not enlarged in 2 (33.3%) only. The values for HBsAg negative group were 15 (62.5%) and 9 (37.5%) respectively. The values are statistically not significant ($\chi^2 = .5$, d.f. = 1, $p < 0.5$)

which indicate that presence or absence of splenomegaly has no bearing regarding the HBsAg positivity in a case of chronic active hepatitis.

Hepatomegaly was present in 3 (50.0%) cases of HBsAg positive group and absent in rest 3.(50%) cases. The values for HBsAg negative group were 11 (45.8%) and 13 (54.2%) respectively. These values are statistically insignificant ($X^2 = .410$, d.f.=1, $p > 0.5$). These data point that presence or absence of hepatomegaly do not govern the HBsAg positivity in chronic active hepatitis case.

Out of the 6 cases who showed the presence of Shikata cell, only 1 (16.7%) case showed signs of hepatic encephalopathy while it was absent in 5 (83.3%) cases. The values for negative group were 9 (37.5%) and 15 (62.5%) respectively. The values are statistically insignificant ($X^2 = .610$, d.f. = 1, $p \leq 0.5$) which indicate that hepatic encephalopathy has no bearing regarding the positivity of HBsAg.

SHIKATA CELL IN RELATION OF VARIOUS LIVER FUNCTION TESTS (BIOCHEMICAL PROFILE) OF DIFFERENT SUB GROUP:

(A) CIRRHOSIS:

The table VIII shows bio-chemical profile in

relation of Shikata cell in cirrhosis cases.

Of the 14 HBsAg positive cases of cirrhosis, the serum bilirubin levels ranged between 0.5 - 1.4 mg.% with a mean of 1.02 mg.% while in the HBsAg negative group the range was 0.5 - 50 mg.% and mean 4.35 mg.%. These values are statistically highly si nificant ($t = 5.80$, d.f., $= 38$, $p < 0.001$) which indicate serum bilirubin levels have definitely a bearing regarding the HBsAg positivity in cirrhosis cases.

TABLE VIII

Cirrhosis (Biochemical Profile)
LIVER FUNCTION TEST ($n = 49$)

	HBsAg positive	HBsAg negative
Serum Bilirum (mg.%)		
Range	.5-1.4	.5-50
Mean	1.02	4.35
Total serum Protein (gm.%)		
Range	5.2-7.8	4.8-8.5
Mean	6.71	6.59
S. Albumen (gm.%)		
Range	2.8-4.3	1.7-4.0
Mean	3.28	2.96
S.Globulin (gm.%)		
Range	2.2-4.6	2.7-5.5
Mean	3.43	3.60
S.G.O.T. (I.U.)		
Range	5-60	11-67
Mean	24.9	31.3

	HBsAg positive	HBsAg negative
S.G.P.T. (I.U.)		
Range	2-24	7-150
Mean	12	18.5
S.Alk phosphatase (KAW)		
Range	2.8-12.5	2.0-31.2
Mean	5.15	8.41

The total serum protien levels ranged between 5.2-7.8 gm.% with a mean of 6.71 in HbsAg positive group while the values in HBsAg negative group were 4.8 - 8.5 gm.% and 6.59 gm.% respectively. These values are statistically not significant ($t = 0.401$, d.f. $= 38$, $p < 0.8$), which indicate that levels of total serum protien have no bearing regarding the Shikata cell positivity.

The serum albumen levels were noted ranging between 2.8 - 4.3 gm.% with a mean of 3.28 gm.% in cases who were proved Shikata cell positive. The values for negative group were 1.7 - 4.0 gm.% and 2.96 gm.% respectively. The values are statistically not significant ($t = 1.633$, d.f. $= 38$, $p < 0.2$) which point that levels of serum albumen do not have any influence regarding the HBsAg positivity.

Out of the 14 Shikata cell positive cases of cirrhosis, a mean serum globulin value came to be 3.43 gm.% with a range of 2.2 - 4.6 gm.% while it was 3.60 gm.% and 2.7 - 5.5 gm.% respectively among the 24 HBsAg negative cirrhotics. These values are statistically insignificant ($t = 0.748$, $d.f. = 38$, $p. > 0.5$). These data too indicate that levels of serum globulin have no influence upon the Shikata cell positivity.

The levels of Serum, glutamic oxalo acetic acid transaminase (S.G.O.T.) ranged between 5 - 60 I.U. in HBsAg positive group with a mean of 24.9 I.U. while the values in HBsAg negative group were 11-67 I.U. and 31.3 I.U. respectively. The values are statistically insignificant ($t = 1.265$, $d.f. = 38$, $p. > 0.2$) which point that S.G.O.T. levels cannot be taken as a point to differentiate between HBsAg positive and HBsAg negative group.

In the group of 14 HBsAg positive cirrhotics, the serum Glutamic pyruvic transaminase (S.G.P.T.) levels ranged between 2-24 I.U. with a mean of 12 I.U. while in the HBsAg negative group they were 7 -150 I.U. and

and 18.5 respectively. The values are statistically insignificant ($t = 0.933$, d.f. $= 38$, $p < 0.5$). The data indicate that levels of S.G.P.T. do not have any bearing regarding the positivity of HBsAg.

Serum alkaline phosphat are values ranged between 2.8 - 12.5 K.A.U. with a mean of 5.15 K.A.U. in HBsAg positive group while they were 2.0 - 13.2 K.A.U. and 8.41 K.A.U. respectively. These data are statistically insignificant ($t = 1.63$, d.f. $= 38$, $p > 0.1$). The data point that levels of serum alkaline phosphate have no bearing regarding the Shikata cell positivity.

(B) CHRONIC ACTIVE HEPATITIS:

The table IX shows the bio-chemical profile in relation of Shikata cell in chronic active hepatitis cases.

TABLE IX

BIOCHEMICAL PROFILE OF CHRONIC ACTIVE HEPATITIS IN RELATIONSHIP OF SHIKATA CELL. (n=30)

	HBsAg positive	HBsAg negative
1. Serum Bilirubin (mg.%)		
Range	1.4 - 2.5	.5 - 3.6
Mean	2.65	.83

Contd.

	HBsAg positive	HBsAg negative
2. Total Serum Protiens (gm.%)		
Range	5.8 - 7.7	5.8 - 8.5
Mean	6.75	6.52
3. Serum Albumen (gm.%)		
Range	1.0 - 4.7	2.8 - 5.1
Mean	3.62	3.17
4. Serum globulin(gm.%)		
Range	1.0 - 4.7	2.8 - 5.1
Mean	3.15	3.33
5. S.G.O.T. (I.U.)		
Range	26 - 60	12 - 111
Mean	40.17	25.29
6. S.G.P.T. (I.U.)		
Range	13 - 42	2 - 98
Mean	21.50	16.83
7. Serum alkaline phosphotase (K.A.U.)		
Range	3.0 - 53.5	2.5 - 16.0
Mean	13.81	4.67

The values of serum bilirubin in the HBsAg positive group comprising of 6 cases, ranged between 1.4 - 2.5 mg.% with a mean of 2.65 mg.% and the corresponding values in HBsAg negative group were 0.5 - 3.6 mg.% and 0.83 mg.% respectively. The values

are statistically highly significant ($t. \pm 4.009$, d.f. & 28, p. < 0.001). These data indicate that levels of serum bilirubin have a positive bearing regarding the Shikata cell positivity in a case of chronic active hepatitis.

The total serum protien levels in the Shikata cell positive group ranged between 5.8 - 7.7 gm.% with a mean of 6.75 gm.% while the similar values in Shikata cell negative group were 5.8 - 8.5 gm.% and 6.52 gm.% respectively. The values are statistically insignificant ($t = 0.275$, d.f. = 28, p. < 0.8), which point that levels of serum protiens do not have any bearing regarding Shikata cell positivity.

The serum albumen levels ranged between 1.0 - 4.7 gm.% with a mean of 3.62 gm.% in Orcein positive group while in the orcein negative group it was 2.8 - 5.1 gm.% and 3.17 gm.% respectively. The values are statistically insignificant ($t = 1.515$, d.f. 28, $p < 0.2$) indicating that serum albumen levels do not have any bearing regarding the orcein positivity.

The serum globulin levels, in the cases who showed presence of Shikata cell, had a mean of 3.15 gm.% with a range of 1.0 - 4.7 gm.% while the values in Shikata

cell negative group were 3.33 gm.% and 2.8 - 5.1 gm.% respectively. The values are statistically not significant ($t = 0.677$, $d.f. = 28$, $p. > 0.5$) pointing that serum globulin levels do not have influence on Shikata cell positivity.

The serum Glutamic oxalo acetic acid transaminase (SGOT) levels were ranging between 26 - 60 I.U. having a mean of 42.17 I.U. in HBsAg positive group while in the negative group corresponding values were 12 - 111 I.U. and 25.29 I.U. respectively. The values are statistically not significant ($t = 1.84$, $d.f. = 28$, $p. < 0.1$) indicating that SGOT levels have no bearing regarding Shikata cell positivity.

The mean values of Serum glutamic pyruvic transaminases (SGPT) were 21.5 I.U. in HBsAg positive group with a range of 13 - 42 I.U. while they were 16.83 I.U. and 2 - 98 I.U. respectively in HBsAg negative group. These values are statistically not significant ($t = 0.631$, $d.f. = 28$, $p > 0.5$) pointing that S.G.P.T. level do not have any influence upon Shikata cell positivity.

The serum alkaline phosphotase levels were found to be ranging between 3 - 53.5 K.A.U. with a mean of 13.87 K.A.U. in the orcein positive group and were

2.5 - 16.0 K.A.V. and 4.67 respectively in orcein negative group. The values are statistically significant ($t = 3.302$, $d.f. = 28$, $p < 0.005$) which indicate that levels of serum alkaline phosphotase have bearing regarding Shikata cell positivity in a case of chronic active hepatitis.

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DISCUSSION

DISCUSSION

Cirrhosis has been an ubiquitous clinical phenomenon over the ages but till date no satisfactory clinical aetiological or morphological classification is available. With the endless progress in medical sciences, a number of aetiological factors have been added to the exhaustive list and deleated from time to time.

Cirrhosis has a remarkable socio-enviromental influence regarding its aetiology, i.e. alcoholic (portal) cirrhosis is very common in western countries, while it is quite rare in India (Kashiwal et al 1963). Post necrotic cirrhosis forms the major aetiologic factor in Indian subcontinent than in western countries. Mc Dermott et al (1961) reported an incidence of 30% while Basu (1967) came out with 55%.

Mac Callum in 1951 showed that there were at least two viral agents producing hepatitis having no cross immunity between them which may later on convert into chronic liver disease. Later on, Blumberg et al in 1965 confirmed the above fact

by discovery of Australia antigen which is associated only with large incubation serum hepatitis or virus B hepatitis and not with infectious hepatitis or virus A hepatitis (W.H.O. 1973). However, later on, other modes of transmission have been implicated as well, i.e. sexual (Hersh et al 1971 , Hemigist 1973), Kissing (Villarejos et al 1974), human bite (Mac Quarrie et al 1974) and transplacental (Stevens et al 1975).

The physical properties of this hepatitis B antigen were later on established. Its detection and demonstration was done by Agar gel (Blumberg et al 1965), Complement fixation test (Purcell et al 1969), Electronmicroscopy (Shulman and Baker 1969), Immuneflorescence (Millman et al 1970), Radio-immunoassay technique (Walsh et al 1970), Electrosmodiffusion (Persendorfer et al 1970, and Immune adherance test (Mayumi et al 1971).

There is now an abundance of literature on the relationship of Australia antigen (HB Ag) and chronic liver disease (Fox et al 1969, Wright 1960, Gitnick 1969, Faber et al 1970, Velasko 1970, Dudley et al 1972 , Grab et al 1972 and Sherlock 1975). The demonstration of hepatitis B antigen has been done in

liver cells in cases of chronic hepatitis and cirrhosis by immunoflerescent and electromicroscopy (Millman et al 1969) which has confirmed the aetiological relationship. The above mentioned techniques were very expensive and time consuming and hence were limited to a very few centres and unsuitable for application in large scale studies.

It was in 1974, when Shikata et al were comparing the Au antigen demonstrated by immunoflerescent method and examining the conventional paraffin sections, noticed accidently that, similar structures which were demonstrated by florescein isothiocyanate (FITC) labelled anti-Au serum were stained faintly black by elastica Van Gieson stain. Later it was proved that black colour was due to resorcin-fuchsin and not by Van Geison stain. This lead new dimensions of better staining method for Au antigen in paraffin sections. Amongst the all, orcein stain gave best results which were stable, easily reproducible and was specific for Au antigen and negative for other serum protiens. Later on, a number of modifications of this orcein staining have been given by a number of workers to obtain still better results (Deodhar et al 1975 , Masachika seneba 1982).

Gerber et al (1974) compared orcein with immuno-florescence and immuno-electron microscopy and concluded that orcein staining results are comparable to any sensitive standard technique. Nayak et al (1975) conducted a study in which comparison of immunoflorescence, immuno-peroxidase and orcein staining was done with regard to their specificity and reliability as antigen marker. Author considered to be a good marker of hepatitis B surface antigen and to have certain advantages over immunostaining. The simplicity of orcein technique becomes more relevant for large scale retrospective and prospective studies.

Impressed by the above results of the simple orcein staining technique, the given study was undertaken in Bundelkhand region to work out the incidence of hepatitis B as the causative of cirrhosis by the modification as suggested by Masachika Seneba (1982).

There were total 97 cases of chronic liver diseases i.e., cirrhosis, chronic active hepatitis, chronic persitant hepatitis, hepatoma and Indian childhood cirrhosis, studied in the present series. The higher frequency of former two diseases, i.e., 49 cirrhosis and 36 chronic

active hepatits, were studied in detail as chronic active hepatitis is a precursor of cirrhosis (Medline et al 1976) and may be assumed to be a changing phase of the same disease process. Although the hepatoma forms the last phase of this chronic liver disease, but in the present study it was diagnosed histopathologically in only 3 cases.

There has been a wide variation regarding the positivity of Shikata cell in cirrhosis in India, due to variations in the prevalence rate of hepatitis B in different geographical areas. Sama et al (1973) reported 59.2%, while Chadda et al (1982) gave 16.7% results only. In the present study the total percentage of orcein positivity in chronic liver disease was found to be 27.83%. Using same technique Aikat et al (1977) reported positivity of 13% in their biopsied cases and 45% in autopsied cases. Chadda et al (1982) reported 16.7% percentage while Priyadarshini et al (1979) concluded with 32.6% positivity. Seth et al (1975) reported a positivity of 37% in needle biopsy cases while it was 78% and 80% in wedge biopsy and autopsy cases, respectively. The difference in the percentage of positivity of Shikata cells in the present study with others can be attributed to the variation in

the prevalence of hepatitis B infection among different geographical where these studies have been conducted.

Cirrhosis could occur at any age but the maximum instance 55% was seen in young adults to middle-aged (16-45 years) in this study. This high incidence in this age group is very well comparable with the observations made by Heathcote et al (1974) that acute hepatitis B tend to affect a slightly older age group (peak being 20-30 year age group) than does type A and so automatically the chronic liver disease is more expected in this age group.

In the cirrhosis sub-group 51% belonged to the age group of 16-45 years and 43% in above 46 years age group while in the chronic active hepatitis sub-group, they were 75% and 22% respectively. These observation compare well with the observation of Heathcote et al (1974) as mentioned above.

In the present study a positivity of HBsAg was found to be 32.69% in cirrhosis sub-group. Seth et al (1975) have reported a 33% positivity while Priyadarshini et al (1979) have reported 36.1% in cirrhotics using same orcein technique. The results of these workers correlate with the observations of present study. Chadda et al (1982) and Aiket et al (1977) reported a lower percentage of 22% and 23% positivity in their study.

prevalence of hepatitis B infection in these geographical areas as also evident by a low total positive percentage reported by the same worker.

In the chronic active hepatitis subgroup of the present study, 19.44% Shikata cell positivity was noted. These results are in conformity with those of Aikat et al (1977) who reported a positivity of 19% in biopsy cases u ing same technique. Chadda et al (1982) have reported only 12.5% positivity which may be attributed to lower prevalence of the disease in that area.

In the Indian Childhood cirrhosis subgroup a positivity of 50% was seen in present study which compares well with 40% positivity reported by Chadda (1982) using the same orcein technique, however, the sample size in the present study was very small. Chandra (1970) reported a still lower, i.e., 20% only which may be because of less prevalence of hepatitis B in that area or due to sampling error.

In the hepatoma sub-group, the positivity noted was 33%, which is higher in comparison to 21.7% reported by Priyadarshini et al (1979). Aikat et al (1977) have reported a positivity of 14% only while it was 4.6% only in a series of Chadda et al (1982).

Okuda et al (1982) reported 31.6% positivity in a series of 426 cases. The discrepancy of results in this study with others may be attributed to small sample size.

In the chronic persistant hepatitis sub-group of the present study, the positivity for Shikata cells was nil. Deodhar et al (1975) reported a positivity of 63.6% in his study of 11 cases only. Carrella et al (1972) have reported a positivity of 16.6% by complement fixation and 11.1% by Immuno-diffusion technique. The nil positivity in the present study may be due to small sample size.

As recommended by Deodhar et al (1975) that this orcein technique is useful for both i.e., in fresh and stored material, the present study included 21 stored material of chronic liver diseases. The results of stored material were equally compafable with those of fresh material and are in agreement with the observation of Deodhar et al (1975).

The observation regarding the clinical and bio-chemical correlation with Shikata cell positivity is not available in almost any of the

work done by other workers. In this series an effort has been made to correlate the Shikata cell positivity with clinical and Bio-chemical profile of the cases of chronic liver disease.

In the cirrhosis sub-group a past history of jaundice, pertioneal tapping, oedema feet, loss of libido or history of immunisation and/or parentral therapy, haematemesis or malena was found to have no bearing regarding the positivity of Shikata cell. All the results, as noted in observations in Table IV while dealing with the symptoms, are statistically not significant except a negative history of immunisation and/or parentral therapy. As 4 (28.6%) of the Shikata cell positive group denied any past history of immunisation or parentral therapy but were positive for HBsAg which concludes that there is some other non-parentral route of infection of serum hepatitis by which these patients were infected. This value is statistically highly significant ($X^2 = 6.013$, d.f. = p <0.02). This finding is comparable with the observations made by other workers who gave a long list of non-sexual (Hersh et al, 1971, Henigst 1973), (Kissing Villarejos et al, 1974), human bite (Mac Quarrie et al, 1974) transplacental (Stevens et al, 1975) sharing

of razors and tooth pastes (Mosley 1975) and blood sucking insects (Prince et al 1972, Zebe et al 1972).

Krugman et al (1967) demonstrated that HBsAg positive serum was infective when given orally as well as parentrally. HbsAg has been sought in virtually all body secretions and has been found in the urine (Tripatzis and Horst, 1971), Saliva (Ward et al, 1972), Semen (Heathcote, Cameron and Dane; 1974) bile (Adamar et al, 1971), breast milk (Boxall et al, 1974), nasal washings (Villarejos et al, 1974), Vaginal secretions (Durani and Gerber, 1974), Sweat (Telatar et al, 1974), Cerebrospinal fluid (Dankert et al, 1975) of subjects with HBsAg in their serum. It remains unknown whether HBsAg in body fluids, other than blood is infective. It is also possible that very often these secretions are contaminated with blood in microscopic amounts. But, undoubtedly, transmission of HBsAg in the absence of overt parentral exposure takes place. Such transmission has been noted to be particularly common between sexual, expecially male homosexual, partners.

As regards the signs of cirrhosis, the observations are charted in Table V of both HBsAg positive and negative group. All the values are statistically not

significant. This confirms that presence or absence of jaundice, anaemia, collateral circulation, ascitis, splenomegaly, hepatomegaly, or hepatic encephalopathy do not have any influence on the Shikata cell positivity. Hence a cirrhosis case cannot be clinically categoriesed into HBsAg positive or negative group.

In the chronic active hepatitis sub-group of the present series, the symptoms as noted, are charted in Table VI. On statistically analysing the result, it was noted that a history of jaundice, peritonial tapping, pedal oedema and immunisation and/or parentral therapy has no bearing regarding the positivity for Shikata cell and the results were statistically not significant. But it was noted that loss of libido was present in much higher percentage of HBsAg positive (83.3%) than in HBsAg negative (54.6%) chronic active hepatitis cases. The results were statistically significant ($X^2 = 5.125$, d.f. 1, $p > 0.02$). A history of haematmesis or malena was present in 33.3% cases of HBsAg positive and only 8.4% cases of HBsAg negative cases of chronic active hepatitis. This is statistically significant ($X^2 = 4.244$, d.f. = 1, $p < 0.05$). This may be concluded that if a patient gives history of haematemesis or malena and

loss of libido then there may be higher chances of its being a HBsAg positive than a HBsAg negative case of chronic active hepatitis. The cause of it is not explainable but the finding is statistically significant.

The observation of the signs of chronic active hepatitis have been given in Table VII. It was noted that jaundice is present in 83.3% cases of HBsAg positive while it was only 8.3% in HBsAg negative group. These values are statistically significant ($\chi^2 = 16.203$, d.f. 28, $p < 0.001$). This may be concluded that jaundice in a histologically proved case of chronic active hepatitis has greater probability of being Shikata cell positive. This corresponds well with the clinical picture of the disease (Sherlock 1975). While the other signs like anaemia, collateral circulation, ascitis, splenomegaly, hepatomegaly and evidence of hepatic encephalopathy do not have any bearing regarding the Shikata cell positivity.

As there is paucity of literature on this particular subject of correlation between Shikata cell positivity with clinical profile of different chronic liver disease, it needs extensive exploration in larger group of patient

in different geographical areas by different workers.

The biochemical status of the cases of cirrhosis sub-group is given in Table VIII. The values on analysing statistically showed that level of serum bilirubin has highly significant relation ($t = 5.80$, $d.f. = 38$, $p < 0.001$). A low level of serum bilirubin was noticed in HBsAg positive cirrhotics and high in HBsAg negative. It is possible that the subsequent attack of viral or serum hepatitis is common in established case of cirrhosis who is HBsAg negative and such recurrent attacks leading to high serum bilirubin level is less common in cirrhosis case which is HBsAg positive. The cause of it is not clear. While the other biochemical profile, i.e., total serum protiens, serum albumen, serum globulin, serum glutamic oxaloacetic acid transaminase, serum glutamic pruvic acid transaminase serum glutamic pruvic acid transaminase and serum alkaline phosphotase do not have any pattern specific with Shikata cell positivity and the results are statistically not significant.

On statistical analysis of the observation of biochemical profile of the chronic active hepatitis

which is mentioned in Table IX, it was inferred that there is significant high level of serum bilirubin in HBsAg positive than in HBsAg negative group. As mentioned in the clinical picture the disease is insideous in onset and the patient feels generally off colour and then notices jaundices (Sherlock 1975). This jaundice which appears in both HBsAg positive and negative group but may last for longer duration in HBsAg positive type and so higher levels have been noticed in this series. But when this converts into cirrhosis in due course of time, the serum bilirubin levels comes to nearly normal levels. This is noted in the present study and confirms that the hepatitis B is a relatively slow disease process than hepatitis A.

The serum alkaline phosphotase level was significantly higher in the HBsAg positive (13.87 K.A.U.) than in HBsAg negative group (4.8 K.A.U.). It may indicate that if higher level is seen in a known case of chronic active hepatitis, chances of its being Shikata cell positive are higher.

No other bio-chemical test showed any significant variation in relation with Shikata cell positivity.

As none of the workers have correlated in such a way, the biochemical correlation of chronic liver disease with Shikata cell positivity, it again requires further explo-nation in this direction. The Shikata cell, which is a marker of previous hepatitis B infection, is seen in the hepatocyte as inclusion body. This HBsAg is rich in cystine (Vyas et al 1972) and thus in disulphide bond and it has been po tulated that the latter are important for antigenicity and particle size of HBsAg (Sukeno et al 1975). The antigen has 6.5 moles of cystine per 100 moles of protien.

The Australia antigen has a great affinity with the orcein dye. The Au antigen seen in the hepatocytes is mainly of three types, i.e., fine granular , powdery, round to oval shape or in clumps (Sachedeva et al 1975). In the present study in the majority of slides, Au antigen was either of round to oval shape or in clumps. Powdery type was seen in very few cases. These took a yellowish or purplish brown stain.

In the present study the distribution of it was noted in cytoplasm only and never inside the nucleus. It was never found in necrotic areas with an inverse relation to the degree of necrosis. This is consistent with the

findings of Dudley et al (1971) and Deodhar et al (1975). The orcein positive material was also seen in Kupffer cells in this study, which correlates well with the observation made by Deodhar et al (1975). The hepatocyte which contained the antigen generally appeared healthy. Similar observation has also been noted by Mayak et al (1975).

In few cases a ground glass appearance of the hepatocyte was also appreciated which were not seen in orcein negative biopsies. This finding is in agreement with the observations made by Deodhar et al (1975). Hadziyannis et al (1973) described initially the ground glass appearance of hepatocyte. It is now believed that deposition of a great excess (more than a million fold) (Maugh 1972) of surface antigen in the cytoplasm is responsible for the so called ground glass appearance of the hepatocyte. This if found in a liver tissue, suggest strong possibility of HBsAg positivity (Hadziyannis et al (1978).

A marked randomness and irregularity was noticed regarding the distribution of the 'Shikata' positive cells in the present study. No predeliction was noted around any vascular territory. These findings are

consistent with the observation made by Nayak et al (1975). Usually positive cells ranged between 2-5% of the hepatocyte (Medline et al (1976) and rarely only 2-5 positive cells were seen in an entire biospy This gives the importance of a thorough scanning of the slide and significance of a positive case.

The error due to randomness of the distribution of Shikata cells may be reduced by examining a bigger tissues. This is the cause that high positivity is reported in wedge biopsy and in autopsy cases as reported by Seth at al (1975) a positive percentage of 37% in needle biopsy cases while it was 78% in wedge biopsy and 80% in antopsy cases. In the present study 95% cases were of needle biopsy and remaining by wedge biopsy.

The high affinity for Au antigen of dyes like orcein, resorcin-fuchsin etc. has been attributed to the multiple disulphide bonds of the An antigen. It is reasonable to presume that aggregate Au antigen gives same structural change or changes of stainability of cytoplasm (Shikata et al 1974) which is not seen in Haematoxylin and Eosin stain and so the detection is possible with orcein or similar stains.

While dealing the orcein dye with liver, a number of

modifications have been given from time to time, to the original technique given by Shikata et al (1974). Deodhar et al (1975) used 0.15 gm. of $KMnO_4$ instead of 0.30 gm., with 0.015 ml. of concentrated sulpheric acid in 100 ml. of distilled water as oxidising solution. The sections were decolourized in 2% oxalic acid for 10 minutes instead of 1.5% used by Shikata and the differntiating medium was 1% acid alcohol instead of absolute alcohol and noted better response as compared to the original technique of Shikata et al (1974).

Masachika Seneba (1982) while using orcein stain noticed that degenerative and necrotic cytoplasm of the hepatocyte was also stained occasionally, hence created difficulty in identification of HBsAg. The author utilised a sensitisor solution (ferric ammonium sulphate or uranium nitrate) after the oxidising solution. When the section is placed in Ferric ammonium sulphate or uranium nitrate solution, prior to the placement in orcein solution, the gaps of protiens are filled with iron or uranium ion. This then left no room for orcein dye and prevented the concurrent staining of the cytoplasm. This gave stable results with better identification of HBsAg. In the present

study this modification was undertaken using ferric ammonium sulphate as sensitiser solution.

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CONCLUSION

CONCLUSION

1. The cirrhosis of liver is quite common in this part of the country which can be well interpreted by the observation that more than 50% of all the chronic liver disease cases were diagnosed as cirrhosis histopathologically.

2. There is no age or sex predeliction for HBsAg positivity in different chronic liver diseases.

3. Orcein staining is simple, easily reproducible with excellent stable results.

4. Shikata cell positivity was present in 27.83% cases of the chronic liver diseases of Bundelkhand region.

5. The positivity for Shikata cells in cirrhosis liver cases was 32.69%, while it was 19.44% in chronic active hepatitis cases.

6. The fact that Shikata cells distribution is random and directly proportional to the amount of the material screened is well known. In the present study 95% cases were of needle biopsy and only 5% of wedge biopsy. With the above

CONCLUSION

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6. The fact that Shikata cells distribution is random and directly proportional to the amount of the material screened is well known. In the present study 95% cases were of needle biopsy and only 5% of wedge biopsy. With the above

fact, the present percentage of Shikata cell positivity is quite significant.

7. The transmission by parentral route of hepatitis B virus was seen in approximately three fourth of the Shikata cell positive cases.

8. The non-parentral route of transmission of hepatitis B virus was also evident in the present study as 28.6% of the Shikata cell positive cirrhotics, denied any past history of possible parentral route of infection.

9. The detection of Shikata cell is easy in fresh as well as stored biopsy material.

10. The technique is quite economical which explores the possibility for mass screening of cases and retrospective analysis to establish the prevalence rate and clinical profile of the disease.

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ANNEXURE I

INCIDENCE OF SHIKATA CELL(HBsAg) IN CIRRHOSIS CASES OF BUNDELKHAND

Name of investigator Dr. Sudhir Mehtotra Case No
MRD No.

Name of patient Address

Age/Sex/Religion Occupation

Date of admission Ward/Bed No.

Consultant Incharge Date of Discharge

CHIEF COMPLAINT

HISTORY OF PRESENT ILLNESS

Fever Anorexia Dyspepsia Weakness

Abdominal pain Jaundice Colour of urine & faeces

Swelling of leg & abdomen Bleeding from nose Skin G.I.T.

Loss of Libido Gynaecomastia

Testicular atrophy Any other

PAST HISTORY

a) Drugs b) Blood Transfusion

c) Immunisation d) Gammaglobulins injection

DIETARY HISTORY

Vegetarian/Non vegetarian High fat/High protein

FAMILY HISTORY

PERSONAL HISTORY

GENERAL EXAMINATION

General condition P.R. R.R. B.P.

Temperature Jaundice Clubbing Anaemia

Cyanosis Hydration White nails Alopaecia

Odema Lymph nodes Skin pigmetation

Parotid Gland Duptryn contracture Palmer erythema

Spider naevae Gynaecomastia Testicular atrophy

Distribution of body hair Foetor

Any other

SYSTEMIC EXAMINATION

Abdomen

Inspection

Visible vein & direction of flow Umblical hernia

Diverication of recti Peristalsic waves

Liver

Size Surface Margin Consistancy

Tenderness other

Gall Bladder

Spleen

Lymph nodes

Evidence of free fluid in abdomen

Testis

Central Nervous System (Signs of hepatic encephalopathy)

Conciousness Sleep Rhythm Personality

Intellectual Function Asterixis

Any evidence of neuro/myelopathy

Genito urinary system

Cardiovascular system

Respiratory system

INVESTIGATIONS

1) Blood

TLC DLC ESR Hb%

BT CT Platelet count

Prothrombin time

2) Stool

Colour Ova/Cyst

3) Urine

Bile salts Bile pigments Urobilinogen

4) Serum

Vanderberg Serum bilirubin

Thymol turbidity Total serum protein

Serum Albumin Serum Globulin

A:G Ratio

5) Enzymes

S.G.O.T. S.G.P.T. S.Alkaline Phosphatase

6) Liver biopsy

7) Staining & demonstration of Shikata cell

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